## Summary

Somatic sensory mechanoreceptors that transduce and convey cutaneous sensations of touch and pain have their cell somas in the dorsal root ganglia (DRG). Despite the obvious importance of cutaneous sensibility, the molecular and physiological mechanisms that underlie mechanoreceptor function are still poorly understood. The goal of my thesis was to identify novel components of the mechanotransduction complex. Three different approaches were used.

In the first project, I searched for a glycosylphosphatidylinositol (GPI)-anchored protein that is involved in mechanotransduction. The idea for this project was based on the finding by Vallet and colleagues that the GPI-anchored serine protease CAP-1 (channel activating protease-1) activates the ion channel ENaC (Vallet et al., 1997). Since ENaC is related to the mechanotransduction channel BNC1, we hypothesized that a related protease may influence BNC1 activity and therefore regulate mechanoreceptor sensitivity. Preliminary studies carried out in chicken embryos by Gary Lewin indeed showed that cleavage of GPI-anchored proteins reduces sensitivity of low threshold mechanoreceptors. My goal was to purify this protein using protein chemistry methods. In protease assays I showed that sensory neurons indeed express a protease that is released after treatment with Phosphatidylinositol-specific phospholipase C (PIPLC), an enzyme that cleaves GPI-anchors. I also showed in binding experiments using the Biacore system, that PIPLC treatment also releases factors that bind to the mechanotransduction channel BNC1. This was in agreement with the working hypothesis that a GPI-anchored protein may interact with BNC1 and modulate it. The binding activity to BNC1, that was determined in Biacore® assays, was used as an assay to detect the protein in further purification steps. Several chromatographic methods were used, but due to strong unspecific binding signals, it was not possible to efficiently purify the protein. For a successful purification in future, a larger amount of starting material and a more reliable assay for the protein would be necessary.

In the main project I took an alternative approach to find mechanotransduction genes. Recent studies revealed that specific subtypes of mechanoreceptors are dependent on neurotrophins. Carroll and colleagues found that BDNF (brain derived neurotrophic factor) heterozygote mice display a reduced sensitivity in slowly adapting (SA) low threshold mechanoreceptors (Carroll et al., 1998). Another study by Stucky and colleagues showed that NT-4 (neurotrophin-4) null mutant mice lose another mechanoreceptor type, D-hair receptors (Stucky et al. 1998). I took advantage of these two mouse models and looked for genes that are downregulated in the DRG (dorsal root ganglia) of these mutant mice. Since these mutant mice do not have any other

accompanying sensory phenotypes except mechanotransduction defects, downregulated genes were good candidates for being involved in these specific mechanoreceptor types. We used Affymetrix® oligonucleotide arrays in combination with a subtracted cDNA libraries to screen for genes that are downregulated in DRG of the mutant mice compared to the WT mice.

The BDNF experiment resulted in 142 genes that were downregulated in the Affymetrix study and 284 genes in the subtracted library. Two genes were common in both groups. In BDNF heterozygote mice, only SA mechanoreceptors comprising a subgroup of large diameter neurons are affected, therefore genes of interest should display a specific expression pattern in large diameter neurons of DRG. DIG-labelled RNA probes derived from a few of the candidate genes were used for in-situ hybridisation, but none of them matched this criterion. Expression patterns of more candidate genes remain to be examined.

In the NT-4 experiment, we wished to find genes expressed only by D-hair mechanoreceptors. D-hair receptors are medium sized DRG neurons with the highest dynamic sensitivity of all sensory neurons innervating skin (Burgess et al., 1968; Brown et al., 1967 a, b; Koltzenburg et al., 1997). Combined analysis of the oligonucleotide microarray study and the subtracted cDNA libraries resulted in 28 candidate genes. Expression studies using in-situ hybridisation revealed that one gene, the T-type calcium channel CaV 3.2, was specifically expressed in medium sized neurons and disappeared almost completely in NT-4 null mutant mice. I therefore concluded that CaV3.2 is specifically expressed in D-hair mechanoreceptors. This is the first description of a molecular marker that is uniquely expressed by a specific mechanoreceptor type. In addition, using an in vitro skin nerve preparation (Koltzenburg et al., 1997) we showed that antagonists acting selectively on T-type calcium channels can block D-hair receptor mechanosensitivity and reduce receptor excitability. We suggest that calcium channels may function to amplify mechanoreceptor responses and thereby specify receptor properties.